



Category: Biology

Type of Paper: Review Article

Received: July 14, 2026, **Revised:** October 15, 2026, **Accepted:** October 20, 2026

Published: October 26, 2026

DOI: [10.54503/0321-1339-2026.126.3-1](https://doi.org/10.54503/0321-1339-2026.126.3-1)

Telomere Maintenance in Cancer: Beyond the Telomerase-versus-ALT Dualism

Meline A. Hakobyan^{1*}

¹Institute of Molecular Biology, Yerevan, 0014, Armenia

*Correspondence: anilem.hakobyan@gmail.com

Abstract

Telomere maintenance is a defining requirement of malignant cells, which must counteract the progressive telomere attrition that otherwise drives replicative senescence. Two principal telomere maintenance mechanisms (TMM) have been characterized: the telomerase-dependent mechanism (TEL), which reactivates the telomerase ribonucleoprotein to add telomeric repeats, and the alternative lengthening of telomeres (ALT), a homologous-recombination-based, telomerase-independent pathway. Historically, these were framed as a mutually exclusive, binary choice inferred largely from canonical genetic lesions such as *TERT* promoter mutations and *ATRX/DAXX* loss. Drawing on mechanistic and structural studies of both pathways, large tumor cohorts that co-measure both mechanisms, and quantitative transcriptomic scoring, this review argues that tumor-level telomere maintenance state is better described as a multidimensional, context-dependent property, varying in the magnitude of each mechanism's activity, in the cellular composition of the tumor, and in temporal stability, than as a binary telomerase-versus-ALT label. This review synthesizes the molecular biology of telomere structure, the architecture and regulation of telomerase, and the recombination machinery underlying ALT, and surveys the current state of the art in telomere-directed therapy. Because telomere maintenance is dynamic rather than fixed, tumors capable of switching between or co-engaging maintenance mechanisms may exhibit reduced sensitivity to therapies targeting a single pathway, supporting TMM-stratified therapeutic assessment.

Keywords: telomeres, telomere maintenance mechanisms, TMM, telomerase, alternative lengthening of telomeres, TMM-targeting therapies

1. Introduction

Telomeres are specialized nucleoprotein structures that cap the ends of linear eukaryotic chromosomes and prevent chromosomal end-to-end fusions and inappropriate activation of the DNA-damage response [1,2]. Because conventional DNA polymerases cannot fully replicate chromosome termini, telomeres shorten with every division in most human somatic cells, and cells with critically short telomeres enter replicative senescence or apoptosis [3–6].

To proliferate indefinitely, cancer cells must therefore reactivate a telomere maintenance mechanism (TMM), which is now regarded as a hallmark of malignancy [7]. Two such mechanisms have been characterized in detail: the telomerase-dependent mechanism (TEL), which reactivates the telomerase ribonucleoprotein [8], and the alternative lengthening of telomeres (ALT), which elongates



telomeres by homology-directed and break-induced replication using telomeric DNA of sister chromatids, non-sister chromosomes, or extrachromosomal telomeric repeats as templates [9,10].

For much of the field's history, TEL and ALT were treated as mutually exclusive states, and a tumor was assigned to one or the other largely on the basis of canonical genetic markers, telomerase activity assays, or telomere-length heterogeneity. However, growing evidence challenges this strict binary view.

In the first report of ALT in human tumors, ALT was assigned only to telomerase-negative samples, yet three telomerase-positive tumors also had ALT-like telomeres, possibly reflecting separate telomerase- and ALT-positive regions or co-existence of both mechanisms within the same cells [11]. In the pan-cancer survey of 31 cancer types, 22% of tumors fell outside both canonical mechanisms, neither expressing *TERT* nor carrying *ATRX* or *DAXX* alterations. A binary TEL-versus-ALT classification therefore leaves a substantial fraction of tumors unassigned [12]. ALT-positive immortalized human cell lines transfected with hTERT sustain telomerase activity alongside recombination-based elongation, showing that the two mechanisms can operate within a single cell in principle [13–15]. In human sarcomas, however, telomerase- and ALT-positive cells occur in the same tumor as separate populations rather than within the same cell [16], so whether co-existence reflects genuine co-engagement or an admixture of two populations remains open. TMM assignment also changes between primary and recurrent tumors in a substantial minority of paired cases [17].

This review surveys the molecular foundations of both mechanisms, the assays through which TMM status is determined and what each can and cannot resolve, the distribution of the two mechanisms across tumor types, and the current state of telomere-directed therapy, and argues from this literature for a graded, context-dependent continuum in place of a binary paradigm.

2. Literature Review and Analysis

Literature search strategy

The literature underlying this review was identified through a staged search of PubMed and Scopus, combining the terms telomere maintenance mechanism, telomerase, hTERT, alternative lengthening of telomeres, ALT, C-circle, ALT-associated PML body, and telomere-directed therapy. A second stage targeted the evidence on which the central argument of this review rests. The terms (“telomerase” OR “TEL” OR “hTERT”) AND (“alternative lengthening of telomeres” OR “ALT”) were combined in turn with “coexistence”, “dual”, “mosaic”, “intratumor heterogeneity”, “switch” and “plasticity”, together with searches for the assays used to establish TMM status (“TRAP assay”, “C-circle”, “ALT-associated PML body”, “telomeric sister chromatid exchange”, “*ATRX*”, “*DAXX*”, “telomere length heterogeneity”). Studies in which both mechanisms were measured in the same samples were retained regardless of publication date, since such studies are comparatively few. A third stage covered therapeutic targeting, searching the agent and target names identified in the preceding stages (“imetelstat”, “BIBR1532”, “6-thio-2'-deoxyguanosine”, “G-quadruplex ligand”, “hTERT vaccine”, “ATR inhibitor”, “FANCM”, “SMARCA1”) in combination with “telomere” or “telomerase”.

Reference lists of retrieved articles and of recent reviews were screened for additional sources. Priority was given to primary studies, to cohorts in which both maintenance mechanisms were measured in the same samples, and, for the mechanistic and therapeutic sections, to work published within the last five years.

Telomere structure and function

The concept of telomeres originated in the late 1930s and 1940s, when Hermann Muller and Barbara McClintock independently recognized that natural chromosome ends behave differently from broken DNA. Muller inferred that chromosome termini are protected from the structural rearrangements affecting broken ends, while McClintock showed that broken ends, unlike natural ones,

fuse and initiate breakage-fusion-bridge cycles [18,19]. Muller later introduced the term telomere, derived from the Greek words “telos” (end) and “meros” (part), to describe these protective chromosome structures [20]. The end-replication problem, articulated independently by Watson and by Olovnikov, explained why conventional DNA polymerases cannot fully copy linear chromosome termini and predicted progressive telomere shortening with each division [21,22]. In the early 1980s, Blackburn and Gall identified the tandem hexanucleotide repeats of telomeric DNA in *Tetrahymena*, and Greider and Blackburn subsequently discovered the telomerase enzyme, providing the first mechanistic solution to the end-replication problem [19,23].

Human telomeres consist of tandem 5'-TTAGGG-3' repeats spanning roughly 5–15 kb in somatic cells and terminating in a 3' G-rich single-stranded overhang of about 50–500 nucleotides [24]. This overhang invades the upstream double-stranded telomeric DNA to form a protective lariat, the T-loop, displacing a strand to create the D-loop and masking the chromosome end from damage-sensing machinery [25,26]. The shelterin complex assembles on telomeric DNA to stabilize the T-loop and to repress inappropriate repair. It comprises six subunits: the double-strand-binding factors TRF1 and TRF2, the single-strand-binding protein POT1, and the connectors TIN2, TPP1, and RAP1. TRF2 protects against inappropriate non-homologous end joining and ATM activation, whereas POT1 coats the single-stranded overhang to repress RPA-dependent ATR signaling (Figure 1) [24,27]. Telomerase recruitment to the 3' end is governed by the TPP1 TEL-patch surface, which engages the enzyme, while TIN2 tethers TPP1 to the double-stranded shelterin core. This recruitment function is separable from end protection, which POT1 provides by coating the single-stranded overhang [28,29]. Overlaid on this protein architecture, G-quadruplex structures formed by stacked guanine tetrads within the G-rich strand impede replication and modulate telomerase access [30,31].

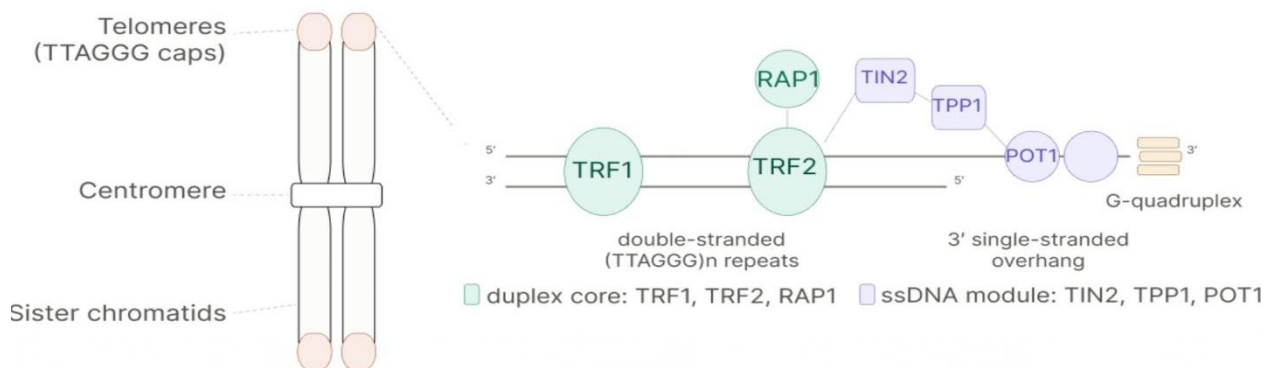


Figure 1. Organization of telomeric DNA and the shelterin complex.

Left: schematic of a replicated chromosome showing the centromere and the telomeric caps at the ends of both sister chromatids. **Right:** enlarged view of a single chromosome end. Human telomeres consist of tandem double-stranded 5'-TTAGGG-3' repeats terminating in a 3' G-rich single-stranded overhang. The six shelterin subunits are shown as two functional modules. The duplex-binding core (green) comprises TRF1 and TRF2, which bind double-stranded repeats directly, together with RAP1, which is recruited through its constitutive interaction with TRF2. The single-stranded module (purple) comprises POT1, which coats the 3' overhang and represses RPA-dependent ATR signaling, and its partner TPP1. The terminal G-rich strand is depicted folded into stacked G-quadruplexes (orange), which impede replication and modulate telomerase access. Original figure created by the author.

More broadly, both telomerase- and recombination-mediated telomere maintenance occur within the context of shelterin-coated chromosome ends, and shelterin regulates telomere accessibility for both processes [32,33]. This shared structural context suggests that shelterin acts as a common regulatory platform influencing telomere maintenance, rather than dedicating the telomere to a single mechanism.



Telomeres and their subtelomeric neighborhoods are enriched in repressive epigenetic marks and associated factors, including H3K9me3, H4K20me3, HP1, and subtelomeric DNA methylation, which contribute to telomere-length control and integrity [34]. Telomeres are also transcribed into the long non-coding RNA TERRA, which forms telomeric R-loops and participates in both protective and recombinogenic processes depending on context [35].

A further consequence of this chromatin environment is the telomere position effect (TPE), the reversible, telomere-length-dependent silencing of subtelomeric genes through the spreading of telomeric heterochromatin [36]. Telomeres can additionally modulate the expression of genes located at considerable genomic distances from the chromosome terminus through long-range chromatin looping, a phenomenon termed telomere position effect over long distances (TPE-OLD), which couples telomere length to the transcriptional output of distal loci [36].

Telomere structure is therefore not a passive backdrop but an actively regulated state, whose chromatin, secondary structure, and transcriptional features vary between cells and tumors [37].

The principal function of telomeres is to preserve genomic stability by distinguishing natural chromosome ends from sites of DNA damage, thereby preventing fusions, recombination, and degradation. By providing an expendable buffer of repetitive sequence, telomeres also protect coding regions from the erosion imposed by the end-replication problem.

Telomere attrition tracks replicative lifespan and links telomere biology to organismal aging [38]. Deviations in either direction are pathogenic: critically short telomeres drive senescence and underlie telomeropathies such as dyskeratosis congenita, whereas pathologically maintained or elongated telomeres support the unlimited proliferation of cancer cells [39,40].

The telomerase-dependent mechanism (TEL)

Telomerase is a ribonucleoprotein reverse transcriptase whose catalytic core comprises the protein subunit hTERT (encoded by *TERT*) and the RNA subunit hTR (encoded by *TERC*), the latter templating the addition of TTAGGG repeats onto the 3' chromosome end [41,42] (Figure 2). The hTERT catalytic subunit is composed of four elements: the telomerase essential N-terminal (TEN) domain, the telomerase RNA-binding domain (TRBD), the reverse transcriptase (RT) domain, and the C-terminal extension. The ~451-nucleotide hTR serves as both template and assembly scaffold, contributing the pseudoknot/template core, the catalytically essential CR4/5 domain, and the 3' H/ACA scaRNA domain that includes the CR7 region [43].

Rather than functioning as a simple on/off switch, telomerase activity is determined quantitatively at three successive levels: the abundance of assembled holoenzymes, the processivity with which the enzyme extends telomeric DNA, and the transcriptional regulation that governs *TERT* expression.

Holoenzyme biogenesis is elaborate and, importantly, dosage-sensitive: the H/ACA proteins dyskerin (DKC1), NOP10 and NHP2 assemble co-transcriptionally on the 3' H/ACA domain of hTR to stabilize the forming RNA, with GAR1 joining during maturation and TCAB1/WRAP53 directing the complex to Cajal bodies. PAPD5 and PARN tune hTR 3' maturation; PAPD5 oligoadenylates the 3' end to target hTR for exosome-mediated degradation, and PARN trims these extensions to yield the stable, mature RNA, thereby setting the steady-state pool of assembled enzyme (Figure 2) [44–46].

Because these steps determine how much functional holoenzyme a cell contains, they constitute the first level at which telomerase output is quantitative rather than all-or-none. Inherited telomere biology disorders illustrate the dosage sensitivity of this level: pathogenic variants affecting *TERC* or *DKC1* reduce the amount or stability of functional telomerase, resulting in progressive telomere shortening whose severity broadly correlates with residual telomerase function [47,48]. Consistent with this, hTR/*TERC* is co-limiting with hTERT in several settings, including cancers with focal *TERC* amplification, so that telomerase output reflects the abundance of both the catalytic (*TERT*) and RNA (*TERC*) subunits rather than the mere presence of hTERT [49,50].

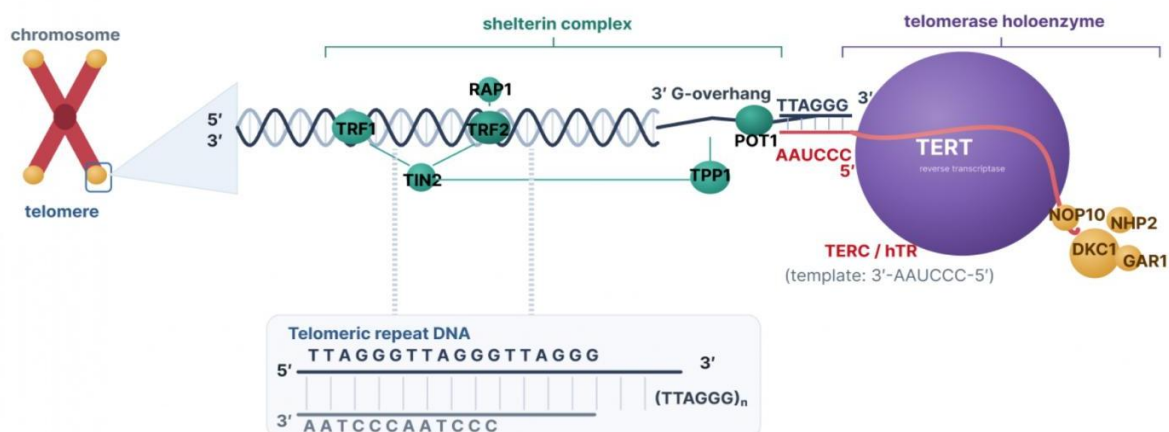


Figure 2. Overview of telomerase-dependent telomere maintenance.

Left: a chromosome expanded into the telomeric duplex; the inset below shows the tandem repeat sequence, with the G-rich strand (5'-TTAGGG-3') paired to the complementary C-rich strand and the 3' G-overhang left unpaired. **Center:** the shelterin complex (green): TRF1 and TRF2 bound to the duplex repeats, RAP1 associated with TRF2, TIN2 linking TRF1 and TRF2 to TPP1, and POT1 bound to the single-stranded overhang. **Right:** the telomerase holoenzyme (purple): the catalytic reverse transcriptase hTERT, and the RNA subunit TERC/hTR (red), whose 3'-AAUCCC-5' template region base-pairs with the 3' terminus of the G-overhang and directs the addition of TTAGGG repeats. The H/ACA-domain-associated proteins dyskerin (DKC1), NOP10, NHP2 and GAR1 (orange), which assemble on hTR and are required for its stability and for holoenzyme accumulation, are shown bound to the RNA. Original figure created by the author.

The past few years have advanced this picture from schematic to near-atomic resolution. Cryo-EM structures of the human telomerase catalytic core, H/ACA lobe, and telomerase bound to the shelterin subunit TPP1 have resolved how the enzyme is assembled, recruited, and activated [44,51]. TPP1 binds two elements of TERT: the TEN domain and the adjacent telomerase RAP motif, stabilizing this interface and enabling efficient telomerase recruitment and activation [51,52]. Structural studies showed that TPP1 and the TPP1-POT1 complex promote telomerase processivity by stabilizing the telomeric DNA substrate and reducing its dissociation during synthesis. These studies also revealed a new DNA-binding path involving the TEN and insertion-in-fingers domain (IFD) domains of TERT [44,51,52]. These structures provide a basis for why telomerase activity is expected to be graded rather than a simple on/off property: RAP is set continuously by the abundance and configuration of the shelterin-associated recruitment factors that engage the enzyme at the telomere. Telomerase does not necessarily extend every telomere in each division; when enzyme availability is limited, it acts sub-saturatingly and with increasing preference for the shortest telomeres [53].

In most somatic cells, *TERT* is transcriptionally silenced, and its reactivation is the rate-limiting step for TEL in cancer. The most frequent mechanism of hTERT reactivation in many cancer types is recurrent somatic mutation of the *TERT* promoter, most commonly the -124 C>T (C228T) and -146 C>T (C250T) substitutions, which create *de novo* ETS transcription factor binding motifs and thereby increase *TERT* transcription [54–56]. The GA-binding protein (GABP) tetramer selectively binds and activates the mutant promoter, accompanied by an allele-specific epigenetic switch to active histone marks and monoallelic *TERT* expression [54,57]. Across 53 cell lines spanning 16 cancer types and six recurrent promoter mutations, the GABPβ1L tetramer was the universal activator of the mutant promoter, yet it was replaceable: its depletion releases an auto-suppression network among GABP subunits, and *TERT* expression is sustained by upregulated GABP dimers or a paralogous tetramer [54,57]. This redundant, self-reinforcing architecture means promoter activity settles at a graded level buffered against perturbation of individual GABP components, a finding with direct implications for promoter-targeted therapy.

Additional factors (MYC, NF- κ B, STAT TFs; repression by CTCF, and by TRF2-bound G-quadruplexes that recruit a REST/PRC2 repressor) further set the quantitative level of telomerase rather than a binary state [55,58].

Taken together, the biogenesis, structural, transcriptional, and functional evidence indicate that telomerase-dependent maintenance is a graded output, set by the amount of assembled holoenzyme, its shelterin-mediated recruitment and processivity, and its transcriptional activity.

Alternative lengthening of telomeres (ALT)

Alternative lengthening of telomeres is a telomerase-independent mechanism that maintains telomeres through homology-directed DNA synthesis, copying telomeric DNA from sister chromatids, non-sister chromosomes, or extrachromosomal telomeric repeats [59].

Two processes have historically been invoked to explain telomere extension in ALT cells: unequal telomeric sister-chromatid exchange (T-SCE), which redistributes existing telomeric DNA between sisters, and homology-directed synthesis, which generates net new telomeric DNA [59]. Current evidence indicates that net lengthening is driven principally by a specialized form of break-induced replication (BIR), in which a resected 3' overhang at a stressed or damaged telomere invades a homologous telomeric template to form a displacement (D-) loop. This nucleates a migrating BIR replisome built on PCNA, RFC, and the POLD3 subunit of DNA polymerase δ (Pol δ), with conservative inheritance of the newly synthesized strand (Figure 3) [60,61]. Elevated T-SCE is now generally regarded as a hallmark of the recombinogenic ALT state rather than an independent lengthening mechanism [59].

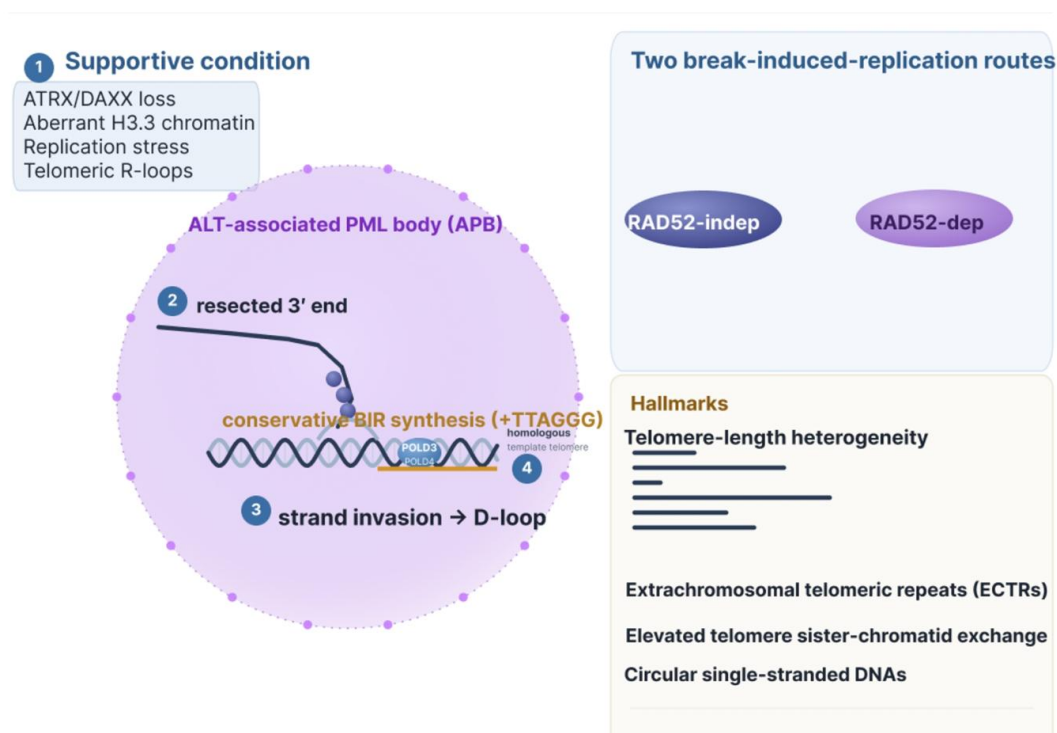


Figure 3. Alternative lengthening of telomeres (ALT) mechanism and characteristic hallmarks.

Left: the sequence of events underlying recombination-mediated telomere synthesis, numbered 1 to 4. **(1)** Permissive conditions listed in the box: loss of *ATRX* or *DAXX*, aberrant H3.3 chromatin, replication stress, and telomeric R-loops. **(2)** Within an ALT-associated PML body (APB), shown as a phase-separated nuclear condensate (purple), a stressed or damaged telomere is resected to expose a 3' single-stranded end. **(3)** The resected end invades a homologous telomeric template to form a displacement (D-) loop. **(4)** A break-induced replication (BIR) replisome incorporating the POLD3 subunit of DNA polymerase δ extends the invading strand, adding new TTAGGG repeats by conservative DNA synthesis. **Top right:** the two genetically separable BIR routes described in the text, RAD52-independent and RAD52-dependent. **Bottom right:** the molecular hallmarks used to recognize the ALT phenotype. Original figure created by the author.



Post-2020 work has resolved ALT into at least two genetically separable BIR routes rather than a single pathway. A RAD52-dependent route drives a substantial fraction of telomere synthesis, whereas a RAD52-independent route, suppressed by RAD51 and MRE11, is associated with elevated C-circle production. Both routes converge on the BLM helicase and the PCNA-RFC-Pol δ replisome [60,61]. Strand invasion itself can proceed through a RAD51-RAD51AP1-TERRA-R-loop-mediated D-loop or through the RAD52-driven annealing pathway, and replication-fork processing factors such as EXD2 help dictate which route is used [61,62]. The RAD52-independent route depends specifically on the fork remodeler SMARCAL1 and the MUS81 nuclease [61].

Synthesis peaks in G2/M and can extend into mitosis as mitotic DNA synthesis (MiDAS) at telomeres [63]. When telomeres remain under-replicated or persistently stressed at the onset of mitosis, DNA synthesis can continue through MiDAS, which in ALT cells frequently relies on RAD52-dependent BIR to complete replication of telomeric DNA [63,64].

The most characteristic hallmark of ALT is the ALT-associated PML body (APB), a phase-separated nuclear condensate enriched with telomeres, shelterin components, and DNA repair proteins. Assembly of APBs is driven by SUMOylation of telomere-associated proteins together with SUMO-SIM interactions, generating a microenvironment that facilitates recombination-dependent telomere DNA synthesis, including BIR [65,66].

The chromatin determinant most frequently associated with ALT is loss of function of the *ATR*X/*DAX*X complex, which deposits the histone variant H3.3 at telomeres and pericentromeric heterochromatin and helps resolve G-quadruplex and R-loop structures. Its loss relieves a repressive constraint on telomeric recombination [67,68].

*ATR*X loss is, however, neither necessary nor sufficient for ALT: many *ATR*X-deficient tumors are not ALT-positive, and ALT can arise with *ATR*X intact, so ALT status is a quantitative, context-dependent outcome rather than a deterministic consequence of a single mutation [69,70].

The ALT phenotype is recognized by a characteristic set of molecular hallmarks: abundant extrachromosomal telomeric repeats, the partially single-stranded C-circles; APBs, in which telomeric DNA, the recombination machinery, and PML co-localise; elevated telomeric sister-chromatid exchange; and pronounced telomere-length heterogeneity, with telomeres ranging from very short to very long [71–73].

Measuring telomere maintenance

The proposition that telomere maintenance exists along a graded rather than binary spectrum must be considered in the context of the methodologies used to define TMM states.

Because the analytical framework determines how TMM activity is quantified and classified, methods that impose binary calls by design may obscure underlying continuous variation, whereas quantitative approaches can capture heterogeneity across the TMM landscape.

Threshold-based assays

The established assays are intrinsically designed to generate binary classifications. Telomerase activity is assayed by the Telomeric Repeat Amplification Protocol (TRAP) against an activity cut-off and complemented by length-based readouts such as terminal restriction fragment analysis (TRF), qPCR, qFISH, and STELA [74]. By these assays, early surveys found the large majority of cancers are telomerase-positive [75]. TRAP reports the presence of telomerase function but does not resolve concurrent or partial engagement of alternative telomere maintenance processes [76]. ALT detection commonly relies on identifying APBs by combined immunofluorescence for PML protein, telomeric DNA FISH [77], and on the C-circle assay, a quantitative method that detects partially single-stranded telomeric DNA circles bearing a continuous C-rich strand [78]. Telomere-length heterogeneity is assessed by telomere-specific FISH or TRF [77], and elevated telomeric sister-chromatid exchange can be scored by chromosome-orientation FISH [61].



Threshold-based assays also differ in what they can resolve spatially, and this difference is independent of their binary output. TRAP, the C-circle assay and TRF analysis are bulk readouts: they report a tumor-level average and cannot distinguish a tumor in which every cell engages both mechanisms from a tumor composed of two separate cell populations. APB immuno-FISH and ATRX/DAXX immunohistochemistry are *in situ* readouts, and chromosome-orientation FISH resolves individual metaphases, although it requires dividing cells and is therefore restricted to cultured material. Each still scores the cell as positive or negative, however, and ATRX/DAXX status reports a permissive genetic lesion rather than telomere maintenance activity itself, which, as noted above, makes it an unreliable proxy in both directions [69].

Interpretation is further limited because no ALT readout is definitive on its own. APBs can assemble in the absence of ongoing telomere synthesis, so their presence does not confirm active elongation, and C-circle signal falls below detection in some tumors that sustain substantial ALT activity [66,79].

All of these approaches dichotomise the output, so intermediate phenotypes are collapsed into a positive or negative call and the underlying continuum is lost before analysis begins.

The consequences are apparent in cohorts where both activities are measured directly. In 412 newly diagnosed gliomas, thresholds of relative telomerase activity ≥ 2 and a C-circle signal above 30% of the U2OS positive control partitioned the cohort into four groups: 36.2% telomerase-positive and ALT-negative, 15.5% ALT-positive and telomerase-negative, 9.5% positive for both, and 38.8% negative for both [17]. The detection of both activities in 9.5% of tumors is inconsistent with a model of strict mutual exclusivity, at the tumor level between TEL and ALT. Tumors negative for both activities exhibited reduced expression of cell-cycle transcriptional programmes and were associated with slower growth of patient-derived lines, suggesting a relatively low-proliferative state rather than providing evidence for a distinct, uncharacterized telomere maintenance pathway [17]. However, threshold-based classification cannot distinguish true absence of detectable maintenance activity from activity below the assay's detection threshold.

Consequently, resolving the biological heterogeneity within this class requires assays that measure telomere maintenance activity on a continuous rather than binary scale.

Quantitative and continuous approaches

Transcriptomic pathway-based approaches are the natural counterpart to threshold-based assays, and they are what make a continuum measurable rather than merely postulated.

Scoring the activity of TEL-associated and ALT-associated pathways from expression data assigns each sample a graded value for each mechanism instead of a label.

Three properties follow. First, the two mechanisms are scored independently, so a sample receives a position in a two-dimensional space rather than a single class, and intermediate states are representable. Second, the score can be computed for a single sample, which makes it applicable to individual clinical specimens rather than only to cohorts. Third, the same computation extends to single-cell expression data, where it can in principle report the relative contribution of the two mechanisms within one tumor and distinguish per-cell co-engagement from an admixture of two populations.

Pan-cancer scoring of ALT-associated activity across TCGA has been used to assign graded ALT scores across tumor types [80]. Transcriptome-wide scoring of TEL-associated and ALT-associated pathway activity across the same resource resolved the 33 cancer types examined into five TMM activity phenotypes, including phenotypes with intermediate and with concurrent activity of both pathways [81]. The pathway scores are continuous, whereas the phenotypes are categories assigned by dividing the score space at two thresholds, identified by segmented regression, into low, middle and high levels [81]. The ALT^{low} TEL^{low} phenotype corresponds to the “neither mechanism” class of the

assay-based studies; within it, tumors retain graded scores for each pathway and can therefore be compared and examined further.

The principal limitation is that these scores are inferred from transcriptional output rather than measured as telomere elongation, and they therefore require anchoring against functional assays in the same samples, which has so far been performed in a limited number of cohorts.

Sequencing-based classifiers form a third quantitative modality and read a continuous feature from routine genome data. Telomere repeat composition differs between ALT-positive and ALT-negative tumors, and classifiers trained on telomeric and variant-repeat content score ALT activity from whole-genome sequencing [82]. Genomic telomere-maintenance footprints have been characterised in the same way across more than 2,500 whole genomes [83], and more recent classifiers extend the approach to sequencing data without matched germline material [84].

Quantitative *in situ* readouts are also emerging and are not captured by the bulk-versus-single-cell distinction alone. Imaging of single-stranded telomeric DNA repeats has been used to quantify ALT-associated activity at the level of individual nuclei [85].

Assays of this type are not yet in wide use, but they address the main gap in the current toolkit, since the established single-cell methods are dichotomising and the established continuous methods are either bulk or inferential.

Table 1. Approaches to determining telomere maintenance mechanism status.

Assay or approach	Input material	Resolution	Output
ATRX/DAXX immunohistochemistry [86]	Tissue sections, FFPE compatible	Single cell, <i>in situ</i>	Binary staining call
APB assay (PML immunofluorescence with telomere FISH) [76,77]	Tissue sections or cultured cells	Single cell, <i>in situ</i>	Percent positive nuclei, thresholded by study-specific criteria
CO-FISH for telomeric sister-chromatid exchange [76]	Metaphase spreads, dividing cells required	Single cell	Exchange events per metaphase, converted to a binary call
TRAP and quantitative TRAP [74,87]	Protein lysate, fresh or snap-frozen only	Bulk	Continuous activity, read against an activity cut-off
C-circle assay [78]	Tumor DNA	Bulk	Continuous signal, read against a cut-off
Telomere length assays (TRF, qPCR, Q-FISH) [74]	DNA, tissue sections, cultured cells	Bulk; single cell for Q-FISH, <i>in situ</i> on sections or per metaphase	Mean length or relative T/S ratio; no direct readout of mechanism
Telomere-composition classifiers [82,83]	Whole-genome sequencing	Bulk	Continuous score, usually thresholded for a call
Transcriptomic pathway scoring [81]	Bulk or single-cell RNA sequencing	Bulk or single cell	Continuous score for each mechanism; categorical phenotypes assigned by thresholds on the scores



No single method resolves mechanism, magnitude and cellular distribution simultaneously (Table 1). Among currently available approaches, transcriptomic scoring provides the most direct representation of graded activity, although the biological calibration of the scores remains open. Neither transcriptomic nor sequencing-based scores measure elongation directly, and both remain anchored to the same threshold-based functional assays whose limitations motivated them. Studies that pair a continuous score with a functional readout in the same specimens are therefore the main requirement for establishing where along the score axis biologically meaningful maintenance begins.

Distribution of telomere maintenance mechanisms across cancer types

At the level of the overall tumor population, the traditional view held that approximately 85–90% of human cancers maintain telomeres through TEL reactivation, whereas ALT is less common and occurs predominantly in tumors of mesenchymal and neuroepithelial origin [88,89].

This distinction originates from the first study to identify TEL-independent telomere maintenance in human tumors and tumor-derived cell lines. Telomeres with the length heterogeneity characteristic of ALT were detected in a subset of these samples lacking measurable telomerase activity, establishing that a minority of cancers can maintain telomeres through an alternative mechanism and raising the possibility that telomerase inhibition would not be uniformly effective [11]. Subsequent prevalence estimates have refined this initial distinction, but their reported frequencies remain dependent on the biological definition and assay used to identify ALT.

Large-scale genomic surveys have substantially refined this picture. In an analysis of 6,835 samples spanning 31 cancer types, *TERT* expression was detected in approximately 73% of cancers and was associated with promoter mutations, rearrangements, amplifications, fusions, and promoter methylation [12]. A further 5% of tumors exhibited undetectable *TERT* expression together with *ATRX* or *DAXX* alterations, elongated telomeres, and elevated TERRA levels, consistent with ALT [12]. The remaining 22% lacked detectable *TERT* expression and showed no *ATRX/DAXX* alterations according to the criteria applied [12]. This distribution, in which a substantial subset of tumors lacks detectable markers of either telomere maintenance mechanism, indicates that the traditional binary classification of TEL- versus ALT-dependent cancers oversimplifies the biological heterogeneity of telomere maintenance.

This heterogeneity becomes even more apparent when telomere maintenance mechanisms are examined across different cancer types. Telomerase activation predominates in most epithelial malignancies, and the pan-cancer resources provide the firmest basis for that generalisation: *TERT* expression is detected in the large majority of carcinomas, and carcinomas are correspondingly depleted of *ATRX/DAXX* truncation and of the genomic telomere features that accompany it [12,83]. Individual series in lung, colorectal, breast and endometrial carcinoma are consistent with this pattern [90–93].

A frequently cited range of 5–15% across cancers is a composite drawn from series that applied different ALT criteria, including APB immunofluorescence, terminal restriction fragment heterogeneity and the C-circle assay, and that are weighted towards the mesenchymal and neuroepithelial lineages in which ALT is enriched [94]. The lower figure of 3.73% derives from a single broad survey of 6,110 primary tumors across 94 cancer subtypes, in which ALT was assessed in fixed tissue by combined telomere-specific FISH and PML immunofluorescence. ALT was identified on the basis of the characteristic bright, heterogeneous telomeric foci, with PML colocalization used to demonstrate ALT-associated promyelocytic bodies in representative cases. No ALT-positive cases were detected among the benign neoplasms or normal tissue samples included in the study [89]. The higher range aggregates permissive and heterogeneous criteria over a sample of tumor types enriched for ALT, whereas the lower range applies one stringent *in situ* criterion uniformly across an unselected series and behaves accordingly as a floor.



Each estimate inherits the limitations of the assay behind it, set out above. *In situ* scoring on tissue microarrays interrogates a small area of each tumor, so focal or regionally restricted ALT can be missed; because ALT-positive and TEL-positive cells are distributed unevenly within tumors, a single core need not represent the lesion. The C-circle assay is more sensitive but is a bulk measurement read against a threshold, and C-circle signal falls below detection in a proportion of tumors that show other evidence of ALT. APB detection reports the presence of the recombination-permissive compartment rather than ongoing elongation. *ATR*X or *DAX*X status, used as a proxy in several genomic surveys, is unreliable in both directions [69], so prevalence figures derived from mutation status alone are not interchangeable with those derived from functional assays.

Among sarcomas, reported ALT frequencies in osteosarcoma differ between cohorts. In a series of pediatric sarcomas assessed by the C-circle assay, 62 of 87 primary osteosarcomas (71%) were C-circle-positive, whereas Ewing sarcoma is ALT-negative (0 of 94), and rhabdomyosarcoma is positive only at low frequency (~3%) [95].

A larger C-circle-anchored series of 776 primary sarcomas spanning 57 subtypes detected C-circles in only 22 subtypes and in 33% of osteosarcomas, with positivity concentrated in complex-karyotype entities including uterine leiomyosarcoma (66%), soft-tissue leiomyosarcoma (64%), pleomorphic liposarcoma (50%) and undifferentiated soft-tissue sarcoma (42%), while translocation-driven subtypes were largely negative [96].

Intermediate or tissue-specific patterns are also observed: adrenocortical carcinoma, for instance, shows telomerase as the predominant mechanism in 79% of cases, although a small subset of these tumors exhibited ALT either alone or alongside TEL activity, whereas benign adenomas and normal adrenal tissues showed no evidence of active TMMs [97].

The distribution of TMMs also varies with patient age. ALT-associated tumors are over-represented among children and young adults, consistent with the age distribution of the mesenchymal and neuroepithelial entities in which ALT predominates [89,98].

Taken together, these data indicate that TMM distribution is most accurately described at the level of tumor type, molecular subtype, and patient age rather than as a single organism-wide split. Each of these figures nonetheless assigns a whole tumor to a single class, which is a property of how the measurements were made rather than of the tumors themselves.

Telomere maintenance as a continuum: Co-existence, mosaicism and switching

Early studies established that telomeres can be maintained in the absence of telomerase, first in human cell lines immortalized *in vitro* [99] and subsequently in human tumors and tumor-derived cell lines [11], showing that human cancers can rely on either mechanism.

Subsequent studies have shown, however, that co-existence can be defined at different biological levels, including within individual cells [13–15], among distinct cell populations within a tumor [16], and across disease evolution in the same patient [17]. These levels of observation represent different biological phenomena and require different experimental approaches.

The strongest evidence that TEL and ALT are intrinsically compatible derives from engineered cellular systems. Ectopic expression of hTERT in the ALT-positive GM847 cell line generated cells in which TEL activity co-existed with established ALT-associated features, including APBs and marked telomere-length heterogeneity, over extended culture [13]. Similar findings were subsequently reported in two additional ALT-positive cellular backgrounds following telomerase reconstitution [14,15].

Evidence for intratumoral heterogeneity in telomere maintenance comes from studies examining TEL and ALT at single-cell resolution. In 57 high-grade sarcomas, both TEL- and ALT-associated phenotypes were detected within the same tumor in 45.6% of cases, yet the two phenotypes occurred in distinct cellular populations, with no individual cell showing evidence of both mechanisms. Thus, the apparent co-occurrence of TEL and ALT at the tumor level reflected cellular mosaicism rather than



per-cell co-engagement of the two mechanisms [16]. The proportions of ALT- and TEL-positive cells varied markedly among mixed tumors, ranging from approximately 1–47% ALT-positive cells and 2–95% TEL-positive cells, such that tumors spanned a continuum from predominantly TEL-associated to predominantly ALT-associated cellular compositions [16]. Thus, although both mechanisms could coexist within the same tumor, their relative cellular representation varied substantially across tumors, supporting a model in which TMM states are distributed along a continuum of tumor-level compositions rather than restricted to discrete TEL or ALT categories.

The largest study to directly measure TEL and ALT activities in the same glioma samples further illustrates the range of TMM states observed in human tumors. Because both the TRAP and C-circle assays were performed on bulk material, the 9.5% of tumors positive for both activities cannot establish whether TEL and ALT were engaged within the same cells or in distinct cell populations [17]. In 64 paired primary and recurrent tumors, the TMM group changed in 42.2% of cases, with transitions in both directions rather than a consistent shift towards either mechanism [17], indicating that TMM status is not a fixed property of the tumor but can change during progression. Considered together, the cross-sectional spectrum and longitudinal changes support a model in which telomere maintenance exists as a dynamic landscape of tumor states, shaped by the relative representation and activity of different maintenance programmes over time, rather than as two permanently discrete alternatives.

In a mouse T-cell lymphoma model carrying an inducible telomerase reverse transcriptase allele, withdrawal of telomerase from established tumors initially slowed tumor growth and reinstated telomere-dysfunction checkpoints, whereas subsequent tumor outgrowth was associated with acquisition of ALT [100]. This finding provides *in vivo* evidence that loss of telomerase dependence can create selective pressure for the emergence of recombination-based telomere maintenance. That anti-telomerase pressure can promote a shift towards ALT has since been discussed as a general resistance mechanism, although the extent to which it operates in treated patients remains to be established [101,102].

Furthermore, transcriptome-wide scoring of TEL- and ALT-associated pathway activity across 33 TCGA cancer types resolved tumors into five TMM phenotypes, including phenotypes with intermediate activity and with concurrent activity of both pathways [81]. Transcriptomic scoring places tumors continuously across a two-dimensional TEL-ALT space, which the assay-based cohorts above cannot represent; the five phenotypes are categories derived from this space by thresholding.

In somatic cell hybrids formed by fusing ALT-positive cells with telomerase-positive immortal cells, the hybrids were telomerase-positive, and the ALT telomere phenotype was repressed, indicating that at least some TEL-positive immortal cells contain repressors of ALT [103].

State of the art in therapeutic targeting of telomere maintenance

TMM status has been associated with clinical characteristics and course in several tumor types, although the direction and strength of association are context-specific [104–106]. ALT positivity has been linked to distinct outcomes in neuroblastoma, where segmental ALT-associated alterations mark a high-risk molecular subgroup, and to differing prognoses in sarcomas, gliomas, and pancreatic neuroendocrine tumors in various series [94,107]. *TERT* promoter mutations have been reported as markers of poorer outcome in some settings, including subsets of glioma and bladder cancer [108,109].

Therapeutic targeting of telomere maintenance has expanded from efforts to inhibit telomerase to a broader range of approaches aimed at disrupting both TEL-dependent and ALT-dependent telomere maintenance.

Current strategies fall into three groups: telomerase-directed strategies, ALT-directed strategies, and broad DNA-damage-response strategies that may include an ALT-sensitised subset (Table 2).

Telomerase-directed strategies

Imetelstat (Rytelo), a 13-mer lipid-conjugated oligonucleotide that acts as a competitive antagonist of the telomerase RNA (hTR) template, became the first telomerase inhibitor to receive regulatory



approval when the US Food and Drug Administration authorized its use in June 2024 for transfusion-dependent anemia in lower-risk myelodysplastic syndrome (MDS) [110,111]. The approval was based on the randomised, placebo-controlled phase 3 IMerge trial, in which 39.8% of imetelstat-treated patients achieved ≥ 8 -week red-blood-cell transfusion independence versus 15.0% with placebo [111]. The principal drawback of imetelstat is on-target haematological toxicity, with grade 3–4 neutropenia and thrombocytopenia reported in 72% and 65% of imetelstat-treated patients versus 7% and 8% in the placebo arm of the IMerge trial [111].

Table 2. Therapeutic strategies targeting telomere maintenance.

Strategy / Agent	Molecular Target	Class	Development Stage
Telomerase-directed strategies			
Imetelstat (Rytelo) [110,111]	Telomerase RNA (hTR) template	Oligonucleotide telomerase inhibitor	FDA-approved (June 2024): transfusion-dependent anemia in lower-risk MDS
BIBR1532 [112,113]	Telomerase catalytic subunit (hTERT)	Small-molecule telomerase inhibitor	Preclinical
6-thio-2'-deoxyguanosine [116]	Telomerase (telomerase- dependent telomere synthesis)	Telomere-disrupting nucleoside analogue	Early-phase clinical
G-quadruplex ligands (telomestatin, RHPS4, BRACO-19) [118–120]	Telomeric G-quadruplex structures	G-quadruplex-stabilising small molecules	Preclinical
hTERT peptide vaccines (GV1001, UV1) [121,122]	hTERT (immunological)	Therapeutic cancer vaccine (immunotherapy)	Clinical
ALT-directed strategies			
FANCM (FANCM–RMI interaction) [123,124]	FANCM branchpoint translocase / FANCM– RMI	ALT-selective DNA repair vulnerability/replication stress regulator	Preclinical
SMARCAL1 [125,126]	SMARCAL1 fork remodeller	ALT-associated DNA replication stress regulator / DNA repair vulnerability	Preclinical
Broad DNA-damage-response strategies with a possible ALT-sensitised subset			
Camonsertib (RP-3500) [127]	ATR kinase	ATR inhibitor	Early clinical development

Direct catalytic inhibition of telomerase activity by small molecules, exemplified by the non-nucleoside telomerase inhibitor BIBR1532, has demonstrated on-target activity in preclinical models [112,113]. However, despite effectively suppressing telomerase activity *in vitro*, BIBR1532 has not



progressed to clinical application, largely due to poor pharmacokinetic properties that limit its therapeutic potential [58,114,115].

Nucleoside analogues can instead induce acute telomere dysfunction. The 6-thio-2'-deoxyguanosine (6-thio-dG; ateganosine) is selectively incorporated into telomeres in a telomerase-dependent manner, triggering rapid telomere uncapping and DNA-damage responses [116]. It has shown preclinical efficacy and is being explored in early-phase clinical studies, including in combination with immune checkpoint blockade in non-small-cell lung cancer [117].

A structure-directed telomere-targeting strategy exploits the formation of G-quadruplex (G4) secondary DNA structures, which arise in guanine-rich telomeric repeats and can hinder telomerase access as well as replication fork progression. Small molecules that selectively stabilize these structures can therefore interfere with telomere maintenance and genome replication dynamics [31]. Classical preclinical G-quadruplex ligands such as telomestatin, RHPS4, and BRACO-19 promote the stabilization of telomeric G-quadruplexes, leading to telomere uncapping, activation of the DNA damage response, and replication stress [118–120].

Finally, telomerase has also been exploited immunologically. The hTERT-derived peptide vaccines GV1001 and UV1 induce hTERT-specific T-cell responses and have been evaluated alone and in combination with immune checkpoint inhibitors, although definitive improvements in overall survival have yet to be established in randomized trials [121,122]. In parallel, hTERT promoter-driven gene and oncolytic viral therapies have advanced into early-phase clinical studies, whereas strategies targeting the shelterin complex remain largely preclinical [128].

ALT-directed strategies

ALT lacks a catalytic enzyme analogous to telomerase and has therefore been targeted primarily through synthetic-lethal approaches that exploit the elevated telomeric replication stress and DNA repair dependencies characteristic of ALT-positive cells [129].

Disruption of the branchpoint translocase FANCM, or of the FANCM-RMI interaction, induces replication stress and telomere dysfunction preferentially in ALT-positive models and has been proposed as a context-dependent ALT-associated vulnerability [123,124]. Related replication fork remodeling factors such as SMARCAL1, particularly in *ATR*X-deficient glioma, have also been implicated in replication-stress tolerance pathways that may intersect with ALT biology. However, no small-molecule inhibitors targeting these factors have yet reached clinical application [125,126].

Ataxia telangiectasia and Rad3-related (ATR) kinase inhibition: a broad DNA damage response strategy and potential vulnerability in ALT-positive tumors

Inhibition of the ataxia telangiectasia and Rad3-related (ATR) kinase is the most clinically advanced replication-stress-directed strategy, but its dependency profile is not ALT-restricted. ATR functions as a central regulator of the DNA damage response (DDR), sensing DNA damage and replication stress and coordinating downstream signaling pathways that stabilize replication forks, promote DNA repair, and maintain genome stability [130]. ALT positivity has been proposed as one further sensitizing context, but the preclinical evidence is contested. Flynn et al. reported that ALT-positive osteosarcoma lines and glioma stem cells are selectively hypersensitive to the ATR inhibitor VE-821 [131]. A subsequent study using an extended panel of ALT- and TEL-positive lines did not reproduce a general ALT-associated hypersensitivity, and observed no change in ATR inhibitor sensitivity in isogenic cells in which ALT was suppressed by *ATR*X re-expression, attributing the variation instead to cell-line differences unrelated to ALT [132].

The first-in-human phase I trial of the oral ATR inhibitor elimusertib (BAY 1895344) in patients with advanced solid tumors produced partial responses in four patients, all of whose tumors had ataxia telangiectasia mutated (ATM) protein loss and/or deleterious ATM mutations [133], and camonsertib (RP-3500) has been evaluated in an early-phase trial selected on homologous recombination and DDR gene status [127]. In neither case was telomere maintenance mechanism an eligibility criterion, so the



observed responses cannot be attributed to ALT targeting. A 2025 case report described a confirmed clinical and molecular response to camonsertib in a patient with ALT-positive metastatic melanoma, presented as the first clinical instance of ATR inhibition in a molecularly confirmed ALT tumor [134], but this remains a single observation. ATR inhibition is therefore best framed as a broad DDR-directed strategy that may encompass an ALT- or *ATRX*-sensitized subset, rather than an ALT-directed therapy. Establishing ALT status as a predictive biomarker would require prospective evaluation in ALT-stratified cohorts.

The classification of tumors as TEL-positive or ALT-positive underpins not only mechanistic description but also the therapeutic stratification outlined above, in which agents are matched to a presumed dominant maintenance mechanism. Therapeutic approaches may benefit from incorporating the continuum of telomere maintenance states. Binary marker-based classification may overlook tumors with intermediate or mixed phenotypes, in which no single mechanism is exclusively dominant or rate-limiting.

3. Discussion

Although TMMs are traditionally classified into two major categories, telomerase-dependent and alternative lengthening of telomeres, emerging evidence suggests that telomere maintenance capacity may encompass a broader spectrum of biological states. The two-mechanism classification of telomere maintenance is best understood as a product of the assays that created it. The continuum model reflects the heterogeneity observed within individual mechanisms, the variability of TMM-associated molecular features across tumors, and the possibility that cancer cells may exhibit intermediate or context-dependent telomere maintenance phenotypes.

The findings presented throughout this review argue against treating TMM status as a fixed, mutually exclusive property of a tumor. Tumors may contain distinct TEL- and ALT-positive cell populations [16], and TMM status can change during tumor progression [17]. At the same time, these observations do not establish that both mechanisms are simultaneously active within individual tumor cells.

The evidence reviewed here supports a layered framework for interpreting TMM status. Mechanistic identity remains important because TEL and ALT represent biologically distinct routes to telomere maintenance. However, the biological state of a tumor is not fully captured by assigning one of these labels. Activity can vary in magnitude, cellular distribution, and stability over time, so the meaning of a TMM classification depends on both the biological question and the resolution of the measurement [16,17].

Several questions follow directly and remain unresolved. The first is whether individual tumor cells that sustain both mechanisms exist in patient tumors at all, which will require assays that report both mechanisms in the same cell rather than in the same specimen. The second concerns tumors scored negative for both mechanisms, a group that current data cannot resolve into its likely constituents: cells maintaining telomeres by a route not yet characterized, cells using a known route at a level below the detection threshold of C-circle or TRAP assays, and cells dividing slowly enough that measurable maintenance is not required over the interval sampled. These possibilities carry different biological consequences and are presently indistinguishable. The third concerns the basis of switching. Longitudinal change in assigned mechanism is consistent with clonal selection acting on pre-existing heterogeneity, but also with genuine mechanistic conversion, and the contribution of intervening therapy has not been isolated because recurrent samples are collected after treatment. A fourth question arises from an apparent inconsistency in the literature. When ALT-positive cells are fused with normal fibroblasts, the resulting hybrids lose the ALT telomere phenotype and senesce, yet immortal segregants revert to it, indicating that normal cells contain dominant, trans-acting repressors of ALT [103]. Fusion with telomerase-positive immortal cells likewise represses the ALT phenotype [103]. This finding contrasts with evidence that TEL activity and ALT can coexist within the same cell,



raising the possibility that ALT repression is dependent on the cellular context and on the specific complement of regulatory factors present in the hybrid. Resolving this would clarify whether stable co-maintenance is a rare state or a suppressed one.

Functional assays are necessary when direct evidence of mechanism is required; spatial or single-cell approaches are needed to resolve cellular distribution; and quantitative molecular or transcriptomic measures can describe variation that is lost in binary calls. These approaches should therefore be viewed as complementary rather than interchangeable. Future studies that combine orthogonal measurements in the same specimens will be particularly important for determining how continuous pathway-associated states relate to functional telomere maintenance.

The therapeutic relevance of this framework is similarly conditional. Variation in TMM state may influence treatment response and may create opportunities for mechanism-informed intervention, but the current evidence does not justify treating a continuum score or ALT classification as a stand-alone clinical decision marker. Therapeutic approaches targeting telomere maintenance are currently progressing at different stages of development including the telomerase inhibitor imetelstat [135], whereas approaches targeting ALT-associated vulnerabilities remain largely under preclinical investigation [124,125], and a continuous measure of ALT and TEL activity would allow eligibility to be set against a defined threshold rather than a detection call. Mosaic tumors, which contain both cell populations, are least well served by a single-mechanism assignment, since treatment directed at one population leaves the other under no selective pressure.

The evidence increasingly supports viewing telomere maintenance as a dynamic and heterogeneous property rather than a fixed categorical state. Its biological interpretation depends on the extent to which each mechanism is engaged, the fraction of cells in which it operates, its spatial distribution within the tumor, and its stability or evolution over time and under therapeutic pressure. Accordingly, telomere maintenance is best considered a graded and mechanism-stratified property, with measurement strategies selected according to the biological question and the resolution required.

4. Conclusions

Telomere maintenance in cancer is better described as a graded, context-dependent continuum than as a binary choice between TEL and ALT. Both mechanisms are set quantitatively, TEL through the abundance, recruitment, and processivity of its holoenzyme, and ALT through the convergence of permissive chromatin and replication-stress-driven recombination. A single tumor may contain cell populations using each, with the relative contribution shifting over time; whether an individual tumor cell ever sustains both remains unresolved. What follows is a measurement requirement: quantitative, mechanism-stratified assessment of both pathways, spanning bulk, single-cell, and functional readouts, is needed to establish how stably a tumor occupies its position during evolution and treatment, and whether that position predicts response to therapy directed at either mechanism.

References

1. O'Sullivan RJ, Karlseder J. Telomeres: protecting chromosomes against genome instability. *Nat Rev Mol Cell Biol.* 2010;11(3):171–181. <https://doi.org/10.1038/nrm2848>
2. Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet.* 2008;42:301–334. <https://doi.org/10.1146/annurev.genet.41.110306.130350>
3. Zakian VA. Telomeres: The beginnings and ends of eukaryotic chromosomes. *Exp Cell Res.* 2012;318(12):1456–1460. <https://doi.org/10.1016/j.yexcr.2012.02.015>
4. Wu P, Takai H, de Lange T. Telomeric 3' Overhangs Derive from Resection by Exo1 and Apollo and Fill-In by POT1b-Associated CST. *Cell.* 2012;150(1):39–52. <https://doi.org/10.1016/j.cell.2012.05.026>



5. Shay JW, Wright WE. Hayflick, his limit, and cellular ageing. *Nat Rev Mol Cell Biol.* 2000;1(1):72–76. <https://doi.org/10.1038/35036093>
6. Shay JW, Wright WE. Role of telomeres and telomerase in cancer. *Semin Cancer Biol.* 2011;21(6):349–353. <https://doi.org/10.1016/j.semcancer.2011.10.001>
7. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144(5):646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
8. Ju Z, Rudolph KL. Telomeres and telomerase in stem cells during aging and disease. *Genome Dyn.* 2006;1:84–103. <https://doi.org/10.1159/000092502>
9. Zhang JM, Yadav T, Ouyang J, Lan L, Zou L. Alternative Lengthening of Telomeres through Two Distinct Break-Induced Replication Pathways. *Cell Rep.* 2019;26(4):955–968.e3. <https://doi.org/10.1016/j.celrep.2018.12.102>
10. Reddel RR. Alternative lengthening of telomeres, telomerase, and cancer. *Cancer Lett.* 2003;194(2):155–162. [https://doi.org/10.1016/s0304-3835\(02\)00702-4](https://doi.org/10.1016/s0304-3835(02)00702-4)
11. Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nat Med.* 1997;3(11):1271–1274. <https://doi.org/10.1038/nm1197-1271>
12. Barthel FP, Wei W, Tang M, et al. Systematic analysis of telomere length and somatic alterations in 31 cancer types. *Nat Genet.* 2017;49(3):349–357. <https://doi.org/10.1038/ng.3781>
13. Perrem K, Colgin LM, Neumann AA, Yeager TR, Reddel RR. Coexistence of alternative lengthening of telomeres and telomerase in hTERT-transfected GM847 cells. *Mol Cell Biol.* 2001;21(12):3862–3875. <https://doi.org/10.1128/MCB.21.12.3862-3875.2001>
14. Grobelyny JV, Kulp-McEliece M, Broccoli D. Effects of reconstitution of telomerase activity on telomere maintenance by the alternative lengthening of telomeres (ALT) pathway. *Hum Mol Genet.* 2001;10(18):1953–1961. <https://doi.org/10.1093/hmg/10.18.1953>
15. Cerone MA, Londono-Vallejo JA, Bacchetti S. Telomere maintenance by telomerase and by recombination can coexist in human cells. *Hum Mol Genet.* 2001;10(18):1945–1952. <https://doi.org/10.1093/hmg/10.18.1945>
16. Gocha ARS, Nuovo G, Iwenofu OH, Groden J. Human sarcomas are mosaic for telomerase-dependent and telomerase-independent telomere maintenance mechanisms: implications for telomere-based therapies. *Am J Pathol.* 2013;182(1):41–48. <https://doi.org/10.1016/j.ajpath.2012.10.001>
17. Kim S, Chowdhury T, Yu HJ, et al. The telomere maintenance mechanism spectrum and its dynamics in gliomas. *Genome Med.* 2022;14:88. <https://doi.org/10.1186/s13073-022-01095-x>
18. McClintock B. The Stability of Broken Ends of Chromosomes in Zea Mays. *Genetics.* 1941;26(2):234–282. <https://doi.org/10.1093/genetics/26.2.234>
19. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell.* 1985;43(2 Pt 1):405–413. [https://doi.org/10.1016/0092-8674\(85\)90170-9](https://doi.org/10.1016/0092-8674(85)90170-9)
20. Chakravarti D, LaBella KA, DePinho RA. Telomeres: History, Health and Hallmarks of Aging. *Cell.* 2021;184(2):306–322. <https://doi.org/10.1016/j.cell.2020.12.028>
21. Olovnikov AM. A theory of marginotomy: The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol.* 1973;41(1):181–190. [https://doi.org/10.1016/0022-5193\(73\)90198-7](https://doi.org/10.1016/0022-5193(73)90198-7)
22. Watson JD. Origin of concatemeric T7 DNA. *Nature New Biol.* 1972;239(94):197–201. <https://doi.org/10.1038/newbio239197a0>
23. Blackburn EH, Gall JG. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. *J Mol Biol.* 1978;120(1):33–53. [https://doi.org/10.1016/0022-2836\(78\)90294-2](https://doi.org/10.1016/0022-2836(78)90294-2)
24. de Lange T. Shelterin-Mediated Telomere Protection. *Annu Rev Genet.* 2018;52:223–247. <https://doi.org/10.1146/annurev-genet-032918-021921>



25. Doksan Y, Wu JY, de Lange T, Zhuang X. Super-resolution fluorescence imaging of telomeres reveals TRF2-dependent t-loop formation. *Cell*. 2013;155(2):345–356. <https://doi.org/10.1016/j.cell.2013.09.048>
26. Smith EM, Pendlebury DF, Nandakumar J. Structural biology of telomeres and telomerase. *Cell Mol Life Sci*. 2020;77(1):61–79. <https://doi.org/10.1007/s00018-019-03369-x>
27. Lim CJ, Cech TR. Shaping human telomeres: from shelterin and CST complexes to telomeric chromatin organization. *Nat Rev Mol Cell Biol*. 2021;22(4):283–298. <https://doi.org/10.1038/s41580-021-00328-y>
28. Nandakumar J, Bell CF, Weidenfeld I, Zaug AJ, Leinwand LA, Cech TR. The TEL patch of telomere protein TPP1 mediates telomerase recruitment and processivity. *Nature*. 2012;492(7428):285–289. <https://doi.org/10.1038/nature11648>
29. Sandhu R, Tricola GM, Lee SY, Tran A, Lazzerini Denchi E. Control of telomerase recruitment and end protection by independent shelterin components. *Nat Commun*. 2026;17(1):1733. <https://doi.org/10.1038/s41467-026-68433-0>
30. Gellert M, Lipsett MN, Davies DR. HELIX FORMATION BY GUANYLIC ACID. *Proc Natl Acad Sci U S A*. 1962;48(12):2013–2018. <https://doi.org/10.1073/pnas.48.12.2013>
31. Robinson J, Raguseo F, Nuccio SP, Liano D, Di Antonio M. DNA G-quadruplex structures: more than simple roadblocks to transcription? *Nucleic Acids Res*. 2021;49(15):8419–8431. <https://doi.org/10.1093/nar/gkab609>
32. Zhang Y, Hou K, Tong J, et al. The Altered Functions of Shelterin Components in ALT Cells. *Int J Mol Sci*. 2023;24(23):16830. <https://doi.org/10.3390/ijms242316830>
33. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev*. 2005;19(18):2100–2110. <https://doi.org/10.1101/gad.1346005>
34. Blasco MA. The epigenetic regulation of mammalian telomeres. *Nat Rev Genet*. 2007;8(4):299–309. <https://doi.org/10.1038/nrg2047>
35. Fernandes RV, Feretzaki M, Lingner J. The makings of TERRA R-loops at chromosome ends. *Cell Cycle*. 2021;20(18):1745–1759. <https://doi.org/10.1080/15384101.2021.1962638>
36. Lee KH, Kim DY, Kim W. Regulation of Gene Expression by Telomere Position Effect. *Int J Mol Sci*. 2021;22(23):12807. <https://doi.org/10.3390/ijms222312807>
37. Cacchione S, Biroccio A, Rizzo A. Emerging roles of telomeric chromatin alterations in cancer. *J Exp Clin Cancer Res*. 2019;38:21. <https://doi.org/10.1186/s13046-019-1030-5>
38. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature*. 1990;345(6274):458–460. <https://doi.org/10.1038/345458a0>
39. Robles-Espinoza CD, Velasco-Herrera M del C, Hayward NK, Adams DJ. Telomere-regulating genes and the telomere interactome in familial cancers. *Mol Cancer Res*. 2015;13(2):211–222. <https://doi.org/10.1158/1541-7786.MCR-14-0305>
40. Rossiello F, Jurk D, Passos JF, d’Adda di Fagagna F. Telomere dysfunction in ageing and age-related diseases. *Nat Cell Biol*. 2022;24(2):135–147. <https://doi.org/10.1038/s41556-022-00842-x>
41. Cohen SB, Graham ME, Lovrecz GO, Bache N, Robinson PJ, Reddel RR. Protein composition of catalytically active human telomerase from immortal cells. *Science*. 2007;315(5820):1850–1853. <https://doi.org/10.1126/science.1138596>
42. Schmidt JC, Cech TR. Human telomerase: biogenesis, trafficking, recruitment, and activation. *Genes Dev*. 2015;29(11):1095–1105. <https://doi.org/10.1101/gad.263863.115>
43. Zhang Q, Kim NK, Feigon J. Architecture of human telomerase RNA. *Proc Natl Acad Sci U S A*. 2011;108(51):20325–20332. <https://doi.org/10.1073/pnas.1100279108>
44. Ghanim GE, Fountain AJ, van Roon AMM, et al. Structure of human telomerase holoenzyme with bound telomeric DNA. *Nature*. 2021;593(7859):449–453. <https://doi.org/10.1038/s41586-021-03415-4>



45. Roake CM, Artandi SE. Regulation of human telomerase in homeostasis and disease. *Nat Rev Mol Cell Biol.* 2020;21(7):384–397. <https://doi.org/10.1038/s41580-020-0234-z>
46. Moon DH, Segal M, Boyraz B, et al. Poly(A)-specific ribonuclease (PARN) mediates 3'-end maturation of the telomerase RNA component. *Nat Genet.* 2015;47(12):1482–1488. <https://doi.org/10.1038/ng.3423>
47. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature.* 1999;402(6761):551–555. <https://doi.org/10.1038/990141>
48. Goldman F, Bouarich R, Kulkarni S, et al. The effect of TERC haploinsufficiency on the inheritance of telomere length. *Proc Natl Acad Sci U S A.* 2005;102(47):17119–17124. <https://doi.org/10.1073/pnas.0505318102>
49. Masutomi K, Yu EY, Khurts S, et al. Telomerase maintains telomere structure in normal human cells. *Cell.* 2003;114(2):241–253. [https://doi.org/10.1016/s0092-8674\(03\)00550-6](https://doi.org/10.1016/s0092-8674(03)00550-6)
50. Cristofari G, Lingner J. Telomere length homeostasis requires that telomerase levels are limiting. *EMBO J.* 2006;25(3):565–574. <https://doi.org/10.1038/sj.emboj.7600952>
51. Sekne Z, Ghanim GE, van Roon AMM, Nguyen THD. Structural basis of human telomerase recruitment by TPP1-POT1. *Science.* 2022;375(6585):1173–1176. <https://doi.org/10.1126/science.abn6840>
52. Liu B, He Y, Wang Y, Song H, Zhou ZH, Feigon J. Structure of active human telomerase with telomere shelterin protein TPP1. *Nature.* 2022;604(7906):578–583. <https://doi.org/10.1038/s41586-022-04582-8>
53. Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell.* 2001;107(1):67–77. [https://doi.org/10.1016/s0092-8674\(01\)00504-9](https://doi.org/10.1016/s0092-8674(01)00504-9)
54. Bell RJA, Rube HT, Kreig A, et al. Cancer. The transcription factor GABP selectively binds and activates the mutant TERT promoter in cancer. *Science.* 2015;348(6238):1036–1039. <https://doi.org/10.1126/science.aab0015>
55. Tornesello ML, Cerasuolo A, Starita N, et al. Reactivation of telomerase reverse transcriptase expression in cancer: the role of TERT promoter mutations. *Front Cell Dev Biol.* 2023;11:1286683. <https://doi.org/10.3389/fcell.2023.1286683>
56. Bell RJA, Rube HT, Xavier-Magalhães A, et al. Understanding TERT Promoter Mutations: A Common Path to Immortality. *Mol Cancer Res.* 2016;14(4):315–323. <https://doi.org/10.1158/1541-7786.MCR-16-0003>
57. Stevers NO, Kim S, Yuan JB, et al. The immortality mechanism of TERT promoter mutant cancers is self-reinforcing and reversible. *Mol Cell.* 2025;85(12):2337–2354.e9. <https://doi.org/10.1016/j.molcel.2025.05.026>
58. Guterres AN, Villanueva J. Targeting telomerase for cancer therapy. *Oncogene.* 2020;39(36):5811–5824. <https://doi.org/10.1038/s41388-020-01405-w>
59. Cesare AJ, Reddel RR. Alternative lengthening of telomeres: models, mechanisms and implications. *Nat Rev Genet.* 2010;11(5):319–330. <https://doi.org/10.1038/nrg2763>
60. Zhang JM, Zou L. Alternative lengthening of telomeres: from molecular mechanisms to therapeutic outlooks. *Cell Biosci.* 2020;10(1):30. <https://doi.org/10.1186/s13578-020-00391-6>
61. Muoio D, Fouquerel E. The alternative lengthening of telomeres pathway through a DNA repair lens: mechanism and therapeutic opportunities. *NAR Cancer.* 2025;7(4):zcaf056. <https://doi.org/10.1093/narcan/zcaf056>
62. Broderick R, Cherdyntseva V, Nieminuszczy J, et al. Pathway choice in the alternative telomere lengthening in neoplasia is dictated by replication fork processing mediated by EXD2's nuclease activity. *Nat Commun.* 2023;14(1):2428. <https://doi.org/10.1038/s41467-023-38029-z>



63. Min J, Wright WE, Shay JW. Alternative Lengthening of Telomeres Mediated by Mitotic DNA Synthesis Engages Break-Induced Replication Processes. *Mol Cell Biol*. 2017;37(20):e00226-17. <https://doi.org/10.1128/MCB.00226-17>
64. Barnes RP, Thosar SA, Opresko PL. Telomere Fragility and MiDAS: Managing the Gaps at the End of the Road. *Genes*. 2023;14(2):348. <https://doi.org/10.3390/genes14020348>
65. Min J, Wright WE, Shay JW. Clustered telomeres in phase-separated nuclear condensates engage mitotic DNA synthesis through BLM and RAD52. *Genes Dev*. 2019;33(13-14):814–827. <https://doi.org/10.1101/gad.324905.119>
66. Zhao R, Xu M, Yu X, et al. SUMO promotes DNA repair protein collaboration to support alternative telomere lengthening in the absence of PML. *Genes Dev*. 2024;38(13-14):614–630. <https://doi.org/10.1101/gad.351667.124>
67. Lynskey ML, Brown EE, Bhargava R, et al. HIRA protects telomeres against R-loop-induced instability in ALT cancer cells. *Cell Rep*. 2024;43(11):114964. <https://doi.org/10.1016/j.celrep.2024.114964>
68. Hartlieb SA, Sieverling L, Nadler-Holly M, et al. Alternative lengthening of telomeres in childhood neuroblastoma from genome to proteome. *Nat Commun*. 2021;12:1269. <https://doi.org/10.1038/s41467-021-21247-8>
69. de Nonneville A, Reddel RR. Alternative lengthening of telomeres is not synonymous with mutations in ATRX/DAXX. *Nat Commun*. 2021;12(1):1552. <https://doi.org/10.1038/s41467-021-21794-0>
70. Brosnan-Cashman JA, Yuan M, Graham MK, et al. ATRX loss induces multiple hallmarks of the alternative lengthening of telomeres (ALT) phenotype in human glioma cell lines in a cell line-specific manner. *PLoS ONE*. 2018;13(9):e0204159. <https://doi.org/10.1371/journal.pone.0204159>
71. Londoño-Vallejo JA, Der-Sarkissian H, Cazes L, Bacchetti S, Reddel RR. Alternative lengthening of telomeres is characterized by high rates of telomeric exchange. *Cancer Res*. 2004;64(7):2324–2327. <https://doi.org/10.1158/0008-5472.can-03-4035>
72. Draskovic I, Arnoult N, Steiner V, Bacchetti S, Lomonte P, Londoño-Vallejo A. Probing PML body function in ALT cells reveals spatiotemporal requirements for telomere recombination. *Proc Natl Acad Sci U S A*. 2009;106(37):15726–15731. <https://doi.org/10.1073/pnas.0907689106>
73. Lee J, Sohn EJ, Lee J, et al. Distinct mechanisms underlying extrachromosomal telomere DNA generation in ALT cancers. *Nucleic Acids Res*. 2025;53(15):gkaf771. <https://doi.org/10.1093/nar/gkaf771>
74. Lai TP, Wright WE, Shay JW. Comparison of telomere length measurement methods. *Phil Trans R Soc B*. 2018;373(1741):20160451. <https://doi.org/10.1098/rstb.2016.0451>
75. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer*. 1997;33(5):787–791. [https://doi.org/10.1016/S0959-8049\(97\)00062-2](https://doi.org/10.1016/S0959-8049(97)00062-2)
76. Henson JD, Reddel RR. Assaying and investigating Alternative Lengthening of Telomeres activity in human cells and cancers. *FEBS Lett*. 2010;584(17):3800–3811. <https://doi.org/10.1016/j.febslet.2010.06.009>
77. Claude E, de Lhoneux G, Pierreux CE, et al. Detection of alternative lengthening of telomeres mechanism on tumor sections. *Mol Biomed*. 2021;2:32. <https://doi.org/10.1186/s43556-021-00055-y>
78. Henson JD, Lau LM, Koch S, Martin La Rotta N, Dagg RA, Reddel RR. The C-Circle Assay for alternative-lengthening-of-telomeres activity. *Methods*. 2017;114:74–84. <https://doi.org/10.1016/j.ymeth.2016.08.016>
79. Fasching CL, Neumann AA, Muntoni A, Yeager TR, Reddel RR. DNA damage induces alternative lengthening of telomeres (ALT) associated promyelocytic leukemia bodies that preferentially associate with linear telomeric DNA. *Cancer Res*. 2007;67(15):7072–7077. <https://doi.org/10.1158/0008-5472.CAN-07-1556>



80. Sung JY, Lim HW, Joung JG, Park WY. Pan-Cancer Analysis of Alternative Lengthening of Telomere Activity. *Cancers*. 2020;12(8):2207. <https://doi.org/10.3390/cancers12082207>
81. Hakobyan M, Binder H, Arakelyan A. Pan-cancer analysis of telomere maintenance mechanisms. *J Biol Chem*. 2024;300(6):107392. <https://doi.org/10.1016/j.jbc.2024.107392>
82. Lee M, Teber ET, Holmes O, et al. Telomere sequence content can be used to determine ALT activity in tumours. *Nucleic Acids Res*. 2018;46(10):4903–4918. <https://doi.org/10.1093/nar/gky297>
83. Sieverling L, Hong C, Koser SD, et al. Genomic footprints of activated telomere maintenance mechanisms in cancer. *Nat Commun*. 2020;11(1):733. <https://doi.org/10.1038/s41467-019-13824-9>
84. Bennett D, Wierdl M, Chakraborty S, et al. Tabular foundation model predicts alternative lengthening of telomeres (ALT) and identifies SMARCA1 as a target in ALT-driven cancers. *bioRxiv* 2026.03.04.709438. <https://doi.org/10.64898/2026.03.04.709438>
85. Frank L, Rademacher A, Mücke N, et al. ALT-FISH quantifies alternative lengthening of telomeres activity by imaging of single-stranded repeats. *Nucleic Acids Res*. 2022;50(11):e61. <https://doi.org/10.1093/nar/gkac113>
86. Heaphy CM, de Wilde RF, Jiao Y, et al. Altered Telomeres in Tumors with ATRX and DAXX Mutations. *Science*. 2011;333(6041):425. <https://doi.org/10.1126/science.1207313>
87. Kim NW, Piatyszek MA, Prowse KR, et al. Specific association of human telomerase activity with immortal cells and cancer. *Science*. 1994;266(5193):2011–2015. <https://doi.org/10.1126/science.7605428>
88. Shay JW, Wright WE. Telomeres and telomerase: three decades of progress. *Nat Rev Genet*. 2019;20(5):299–309. <https://doi.org/10.1038/s41576-019-0099-1>
89. Heaphy CM, Subhawong AP, Hong SM, et al. Prevalence of the Alternative Lengthening of Telomeres Telomere Maintenance Mechanism in Human Cancer Subtypes. *Am J Pathol*. 2011;179(4):1608–1615. <https://doi.org/10.1016/j.ajpath.2011.06.018>
90. Dobija-Kubica K, Zalewska-Ziob M, Bruliński K, et al. Telomerase activity in non-small cell lung cancer. *Kardiologia i Torakochirurgia Pol*. 2016;13(1):15–20. <https://doi.org/10.5114/kitp.2016.58959>
91. Bertorelle R, Rampazzo E, Pucciarelli S, Nitti D, De Rossi A. Telomeres, telomerase and colorectal cancer. *World J Gastroenterol*. 2014;20(8):1940–1950. <https://doi.org/10.3748/wjg.v20.i8.1940>
92. Makki J, Myint O, Wynn AA, Samsudin AT, John DV. Expression Distribution of Cancer Stem Cells, Epithelial to Mesenchymal Transition, and Telomerase Activity in Breast Cancer and Their Association with Clinicopathologic Characteristics. *Clin Med Insights Pathol*. 2015;8:1–16. <https://doi.org/10.4137/CPath.S19615>
93. Alnafakh RAA, Adishesh M, Button L, Saretzki G, Hapangama DK. Telomerase and Telomeres in Endometrial Cancer. *Front Oncol*. 2019;9:344. <https://doi.org/10.3389/fonc.2019.00344>
94. MacKenzie D, Watters AK, To JT, et al. ALT Positivity in Human Cancers: Prevalence and Clinical Insights. *Cancers*. 2021;13(10):2384. <https://doi.org/10.3390/cancers13102384>
95. Burrow TA, Koneru B, Macha SJ, et al. Prevalence of alternative lengthening of telomeres in pediatric sarcomas determined by the telomeric DNA C-circle assay. *Front Oncol*. 2024;14:1399442. <https://doi.org/10.3389/fonc.2024.1399442>
96. Biondi N, Ratto ML, Pal R, et al. Alternative lengthening of telomeres stratifies complex karyotype sarcomas into distinct genomic and transcriptomic states. *bioRxiv* 2026.05.17.725728. <https://doi.org/10.64898/2026.05.17.725728>
97. Else T, Giordano TJ, Hammer GD. Evaluation of telomere length maintenance mechanisms in adrenocortical carcinoma. *J Clin Endocrinol Metab*. 2008;93(4):1442–1449. <https://doi.org/10.1210/jc.2007-1840>



98. Grandin N, Pereira B, Cohen C, et al. The level of activity of the alternative lengthening of telomeres correlates with patient age in IDH-mutant ATRX-loss-of-expression anaplastic astrocytomas. *Acta Neuropathol Commun*. 2019;7(1):175. <https://doi.org/10.1186/s40478-019-0833-0>
99. Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR. Telomere elongation in immortal human cells without detectable telomerase activity. *EMBO J*. 1995;14(17):4240–4248. <https://doi.org/10.1002/j.1460-2075.1995.tb00098.x>
100. Hu J, Hwang SS, Liesa M, et al. Antitelomerase therapy provokes ALT and mitochondrial adaptive mechanisms in cancer. *Cell*. 2012;148(4):651–663. <https://doi.org/10.1016/j.cell.2011.12.028>
101. Kumar M, Lechel A, Güneş Ç. Telomerase: The Devil Inside. *Genes*. 2016;7(8):43. <https://doi.org/10.3390/genes7080043>
102. Recagni M, Bidzinska J, Zaffaroni N, Folini M. The Role of Alternative Lengthening of Telomeres Mechanism in Cancer: Translational and Therapeutic Implications. *Cancers*. 2020;12(4):949. <https://doi.org/10.3390/cancers12040949>
103. Perrem K, Bryan TM, Englezou A, Hackl T, Moy EL, Reddel RR. Repression of an alternative mechanism for lengthening of telomeres in somatic cell hybrids. *Oncogene*. 1999;18(22):3383–3390. <https://doi.org/10.1038/sj.onc.1202752>
104. Gao J, Pickett HA. Targeting telomeres: advances in telomere maintenance mechanism-specific cancer therapies. *Nat Rev Cancer*. 2022;22(9):515–532. <https://doi.org/10.1038/s41568-022-00490-1>
105. Costa A, Daidone MG, Daprai L, et al. Telomere maintenance mechanisms in liposarcomas: association with histologic subtypes and disease progression. *Cancer Res*. 2006;66(17):8918–8924. <https://doi.org/10.1158/0008-5472.CAN-06-0273>
106. Sung JY. Alternative Lengthening of Telomeres: A Prognostic Paradox in Cancer. *Cells*. 2025;14(20):1613. <https://doi.org/10.3390/cells14201613>
107. Ackermann S, Cartolano M, Hero B, et al. A mechanistic classification of clinical phenotypes in neuroblastoma. *Science*. 2018;362(6419):1165–1170. <https://doi.org/10.1126/science.aat6768>
108. Rachakonda PS, Hosen I, de Verdier PJ, et al. TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. *Proc Natl Acad Sci U S A*. 2013;110(43):17426–17431. <https://doi.org/10.1073/pnas.1310522110>
109. Powter B, Jeffreys SA, Sareen H, et al. Human TERT promoter mutations as a prognostic biomarker in glioma. *J Cancer Res Clin Oncol*. 2021;147(4):1007–1017. <https://doi.org/10.1007/s00432-021-03536-3>
110. Patel H, Yohannes S, Brunaugh E. Imetelstat: A First-in-Class Telomerase Inhibitor for the Treatment of Patients With Lower-Risk Myelodysplastic Syndromes and Anemia. *J Adv Pract Oncol*. 2025;16(7):1–7. <https://doi.org/10.6004/jadpro.2025.16.7.22>
111. Kim N, Pulte ED, Ehrlich LA, et al. US Food and Drug Administration Approval Summary: Imetelstat for Treatment of Selected Patients with Low- to Intermediate-1 Risk Myelodysplastic Syndromes with Transfusion-Dependent Anemia. *J Clin Oncol*. 2025;43(35):3760–3768. <https://doi.org/10.1200/JCO-25-01369>
112. Lavanya C, Venkataswamy MM, Sibin MK, Srinivas Bharath MM, Chetan GK. Down regulation of human telomerase reverse transcriptase (hTERT) expression by BIBR1532 in human glioblastoma LN18 cells. *Cytotechnology*. 2018;70(4):1143–1154. <https://doi.org/10.1007/s10616-018-0205-9>
113. Doğan F, Özateş NP, Bağca BG, et al. Investigation of the effect of telomerase inhibitor BIBR1532 on breast cancer and breast cancer stem cells. *J Cell Biochem*. 2019;120(2):1282–1293. <https://doi.org/10.1002/jcb.27089>
114. El-Daly H, Kull M, Zimmermann S, Pantic M, Waller CF, Martens UM. Selective cytotoxicity and telomere damage in leukemia cells using the telomerase inhibitor BIBR1532. *Blood*. 2005;105(4):1742–1749. <https://doi.org/10.1182/blood-2003-12-4322>



115. Arndt GM, MacKenzie KL. New prospects for targeting telomerase beyond the telomere. *Nat Rev Cancer*. 2016;16(8):508–524. <https://doi.org/10.1038/nrc.2016.55>
116. Mender I, Gryaznov S, Dikmen ZG, Wright WE, Shay JW. Induction of telomere dysfunction mediated by the telomerase substrate precursor 6-thio-2'-deoxyguanosine. *Cancer Discov*. 2015;5(1):82–95. <https://doi.org/10.1158/2159-8290.CD-14-0609>
117. Muller V, Jankowski T, Kowal-Rosinska M, et al. 564 A phase 2 study of ateganosine (THIO) in combination with immune checkpoint inhibitor (ICI) in patients with advanced NSCLC resistant to prior ICI and chemotherapy: THIO-101 trial in progress. *Journal for ImmunoTherapy of Cancer*. 2025; 13(Suppl 2):A644. <https://doi.org/10.1136/jitc-2025-SITC2025.0564>
118. Burger AM, Dai F, Schultes CM, et al. The G-quadruplex-interactive molecule BRACO-19 inhibits tumor growth, consistent with telomere targeting and interference with telomerase function. *Cancer Res*. 2005;65(4):1489–1496. <https://doi.org/10.1158/0008-5472.CAN-04-2910>
119. Tahara H, Shin-Ya K, Seimiya H, Yamada H, Tsuruo T, Ide T. G-Quadruplex stabilization by telomestatin induces TRF2 protein dissociation from telomeres and anaphase bridge formation accompanied by loss of the 3' telomeric overhang in cancer cells. *Oncogene*. 2006;25(13):1955–1966. <https://doi.org/10.1038/sj.onc.1209217>
120. Phatak P, Cookson JC, Dai F, et al. Telomere uncapping by the G-quadruplex ligand RHPS4 inhibits clonogenic tumour cell growth in vitro and in vivo consistent with a cancer stem cell targeting mechanism. *Br J Cancer*. 2007;96(8):1223–1233. <https://doi.org/10.1038/sj.bjc.6603691>
121. Ellingsen EB, Aamdal E, Guren T, et al. Durable and dynamic hTERT immune responses following vaccination with the long-peptide cancer vaccine UV1: long-term follow-up of three phase I clinical trials. *J Immunother Cancer*. 2022;10(5):e004345. <https://doi.org/10.1136/jitc-2021-004345>
122. Middleton G, Silcocks P, Cox T, et al. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): an open-label, randomised, phase 3 trial. *Lancet Oncol*. 2014;15(8):829–840. [https://doi.org/10.1016/S1470-2045\(14\)70236-0](https://doi.org/10.1016/S1470-2045(14)70236-0)
123. Lu R, O'Rourke JJ, Sobinoff AP, et al. The FANCM-BLM-TOP3A-RMI complex suppresses alternative lengthening of telomeres (ALT). *Nat Commun*. 2019;10(1):2252. <https://doi.org/10.1038/s41467-019-10180-6>
124. O'Rourke JJ, Bythell-Douglas R, Dunn EA, Deans AJ. ALT control, delete: FANCM as an anti-cancer target in Alternative Lengthening of Telomeres. *Nucleus*. 2019;10(1):221–230. <https://doi.org/10.1080/19491034.2019.1685246>
125. Taglialatela A, Lee J, Azeroglu B, et al. SMARCAL1 is a candidate therapeutic target for ALT-positive tumors. *bioRxiv* 2026.02.15.704070. <https://doi.org/10.64898/2026.02.15.704070>
126. Brown A, Strickland LM, Erman EN, et al. SMARCAL1 is a targetable synthetic lethal therapeutic vulnerability in ATRX-deficient gliomas that use alternative lengthening of telomeres. *Neuro-Oncol*. 2026;28(4):895–910. <https://doi.org/10.1093/neuonc/noaf300>
127. Yap TA, Fontana E, Lee EK, et al. Camonsertib in DNA damage response-deficient advanced solid tumors: phase 1 trial results. *Nat Med*. 2023;29(6):1400–1411. <https://doi.org/10.1038/s41591-023-02399-0>
128. Zanetti M. A second chance for telomerase reverse transcriptase in anticancer immunotherapy. *Nat Rev Clin Oncol*. 2017;14(2):115–128. <https://doi.org/10.1038/nrclinonc.2016.67>
129. Pan X, Ahmed N, Kong J, Zhang D. Breaking the end: Target the replication stress response at the ALT telomeres for cancer therapy. *Mol Cell Oncol*. 2017;4(6):e1360978. <https://doi.org/10.1080/23723556.2017.1360978>
130. Saldivar JC, Cortez D, Cimprich KA. The essential kinase ATR: ensuring faithful duplication of a challenging genome. *Nat Rev Mol Cell Biol*. 2017;18(10):622–636. <https://doi.org/10.1038/nrm.2017.67>



131. Flynn RL, Cox KE, Jeitany M, et al. Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors. *Science*. 2015;347(6219):273–277. <https://doi.org/10.1126/science.1257216>
132. Deeg KI, Chung I, Bauer C, Rippe K. Cancer Cells with Alternative Lengthening of Telomeres Do Not Display a General Hypersensitivity to ATR Inhibition. *Front Oncol*. 2016;6:186. <https://doi.org/10.3389/fonc.2016.00186>
133. Yap TA, Tan DSP, Terbuch A, et al. First-in-Human Trial of the Oral Ataxia Telangiectasia and Rad3-Related Inhibitor BAY 1895344 in Patients with Advanced Solid Tumors. *Cancer Discov*. 2021;11(1):80–91. <https://doi.org/10.1158/2159-8290.CD-20-0868>
134. Ngoi NYL, Silverman IM, Johnson A, et al. Exceptional response to the ATR inhibitor, camonsertib, in a patient with ALT+ metastatic melanoma. *NPJ Precis Oncol*. 2025;9(1):227. <https://doi.org/10.1038/s41698-025-01025-1>
135. Platzbecker U, Santini V, Fenaux P, et al. Imetelstat in patients with lower-risk myelodysplastic syndromes who have relapsed or are refractory to erythropoiesis-stimulating agents (IMerge): a multinational, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2024;403(10423):249–260. [https://doi.org/10.1016/S0140-6736\(23\)01724-5](https://doi.org/10.1016/S0140-6736(23)01724-5)

Funding: This research received no external funding.

Conflicts of Interest: The author declares no conflicts of interest.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of REPNAS and/or the editor(s). REPNAS and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.